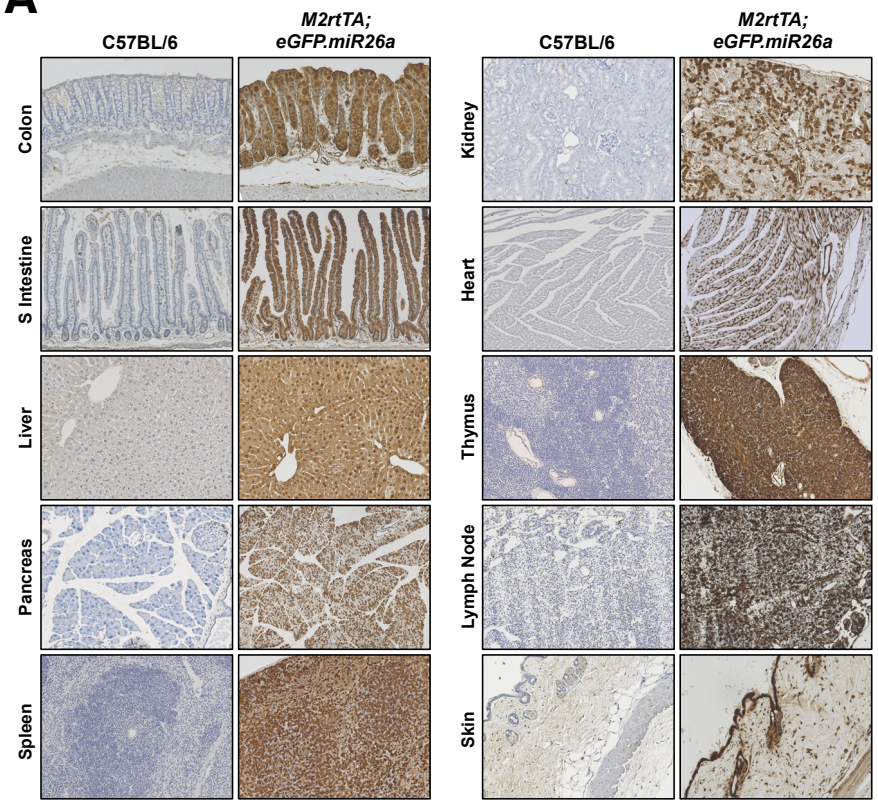
A



B

Tissue	Cell Type	eGFP Expression
Spleen	T cells	+++
	B cells	+++
Thymus	All cells	+++
Lymph Node	T cells	+++
	B cells	+++
Liver	Hepatocytes	+++
Pancreas	Acinar cells	++
	Islets	-
Kidney	Tubular cortex	++
	Collecting duct	-
	Medulla	-
Intestines	Villi	+++
	Crypt	+++
	Mesenchyme	-
Bladder	Epithelial cells	++
Skin	Epithelial cells	+++
	Fibroblasts	+
Lung	Alveolar cells	+
	Broncheolar epithelial cells	+
Heart	Myocytes	+
	Fibroblasts	+
Skeletal Muscle		-
Brain		-

-	No expression
+	<10% of the cells score GFP positive
++	10-50% of the cells score GFP positive
+++	>50% of the cells score GFP positive

Figure S1. Transgene expression in *M2rtTA; eGFP.miR-26a* mice.
A. Anti-GFP immunohistochemistry on tissues from control or dox-treated *M2rtTA; eGFP.miR-26a* mice.
B. Summary of transgene expression based on GFP immunohistochemistry.

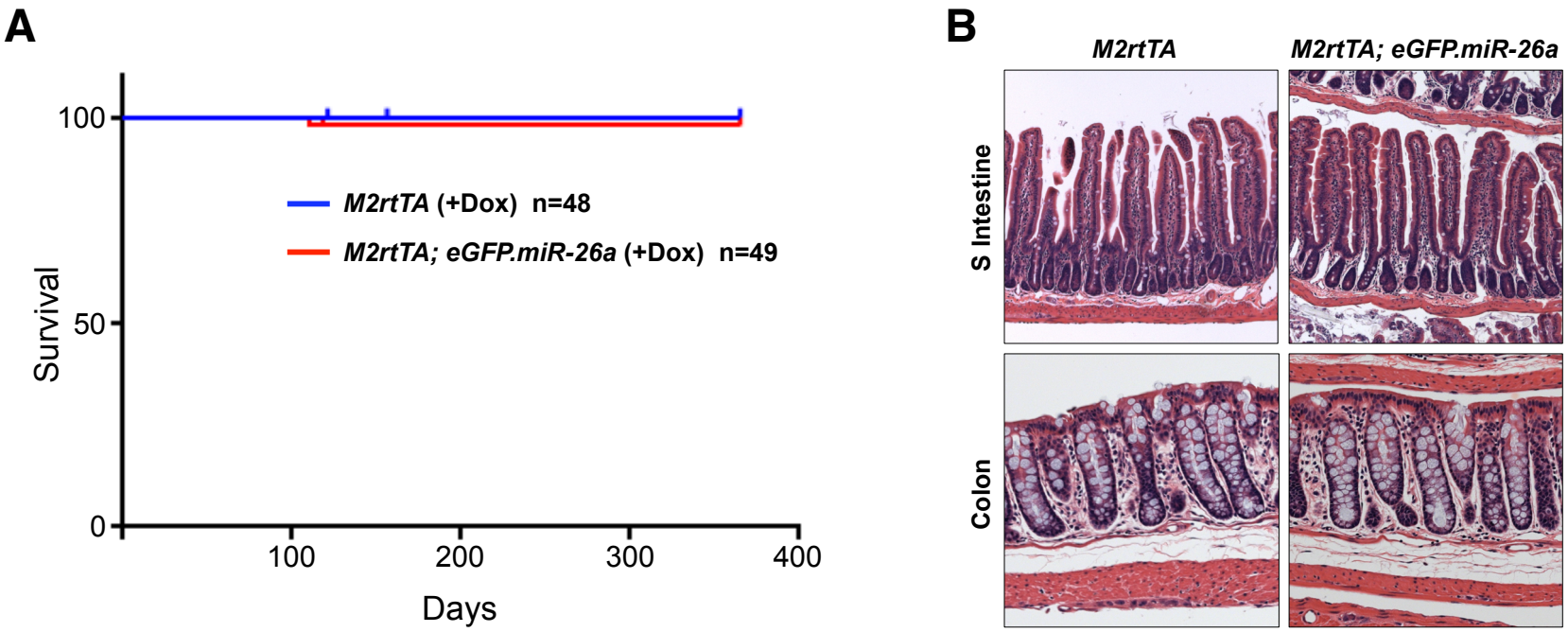


Figure S2. No evidence of malignancy or intestinal abnormalities in *M2rtTA; eGFP.miR-26a* transgenic mice.
A. 1 year survival of doxycycline-treated mice of the indicated genotypes. Dox-treatment was initiated at weaning (28 days of age).
B. H&E-stained sections of small intestine and colon of mice of the indicated genotypes.

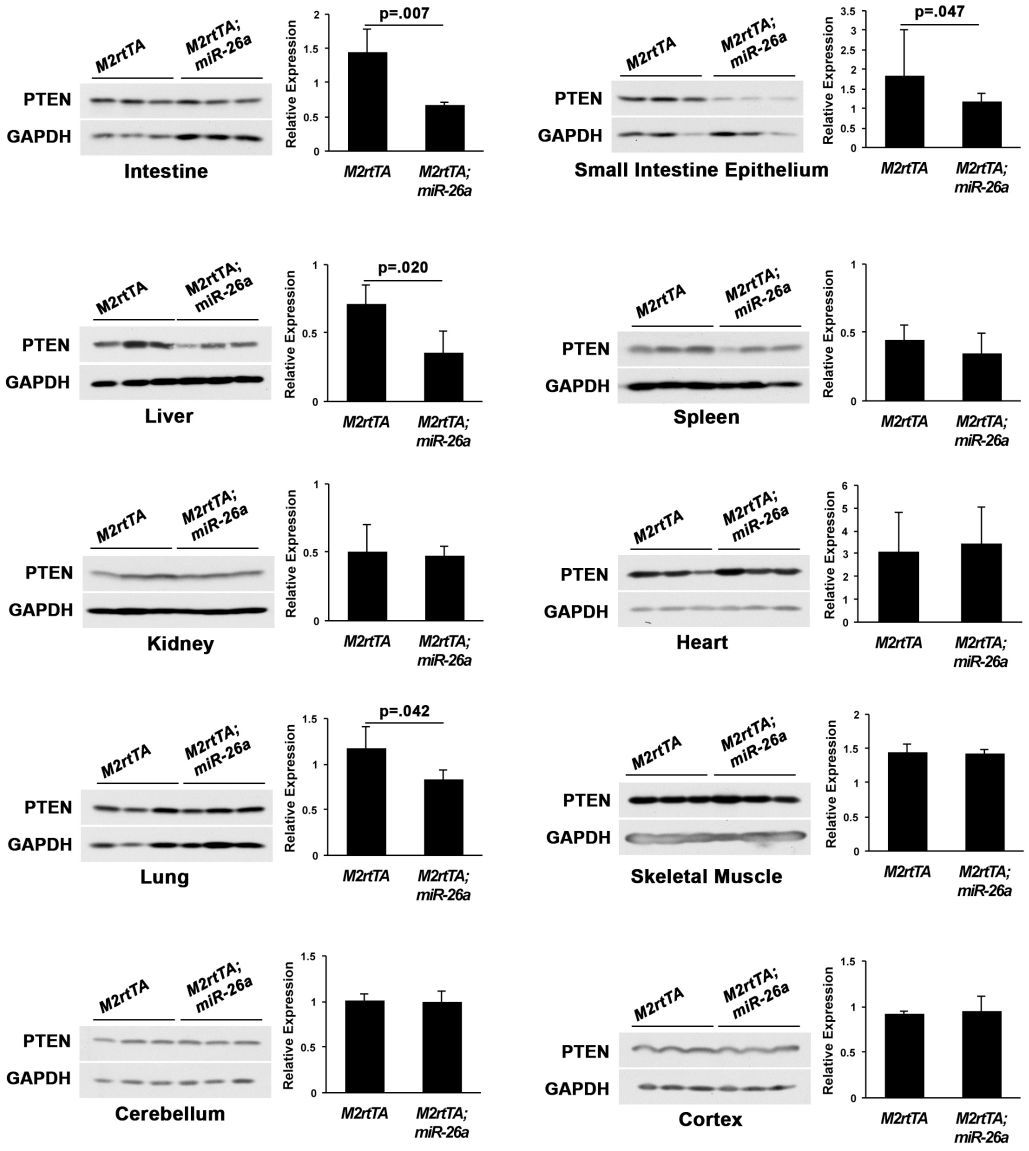


Figure S3. miR-26a represses PTEN *in vivo*. Western blots showing PTEN levels in the indicated tissues of *M2rtTA* or *M2rtTA; eGFP.miR-26a* mice after 2 weeks of dox treatment. Graphs show quantification of PTEN signal normalized to GAPDH. Statistically-significant repression of PTEN expression is indicated by the presence of a p value on graph (2-tailed t-test).

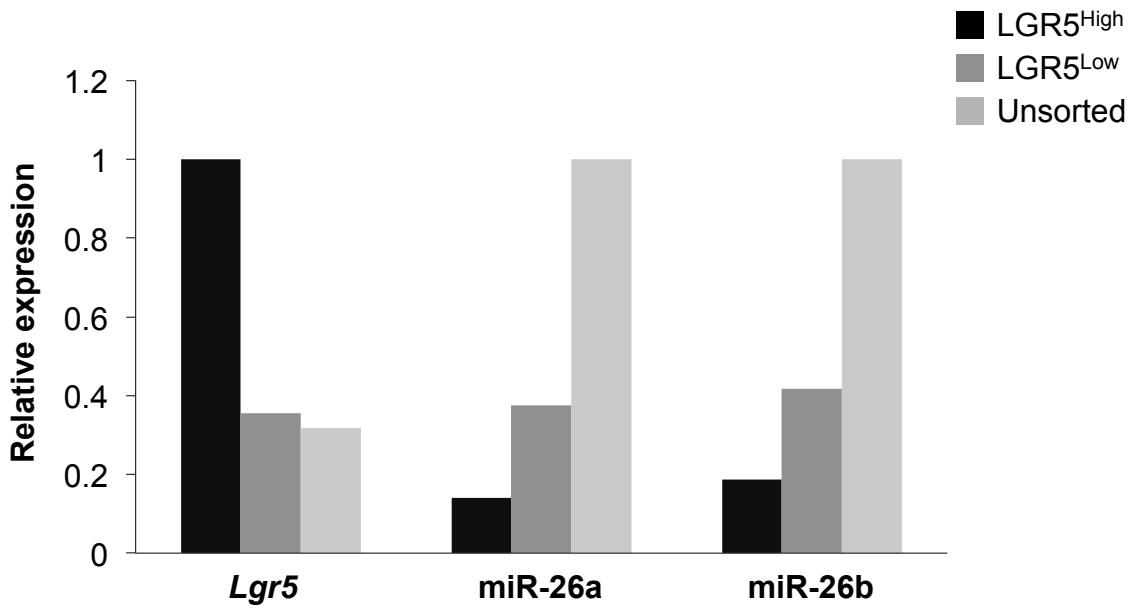


Figure S4. Expression of *Lgr5* and miR-26 family members in intestinal stem cells. LGR5^{High} and LGR5^{Low} cells were sorted from intestinal epithelial cells isolated from *Lgr5*$^{+/eGFP}$ mice (Barker et al. 2007). *Lgr5* expression was normalized to 18S and miR-26a/miR-26b expression was normalized to U6.

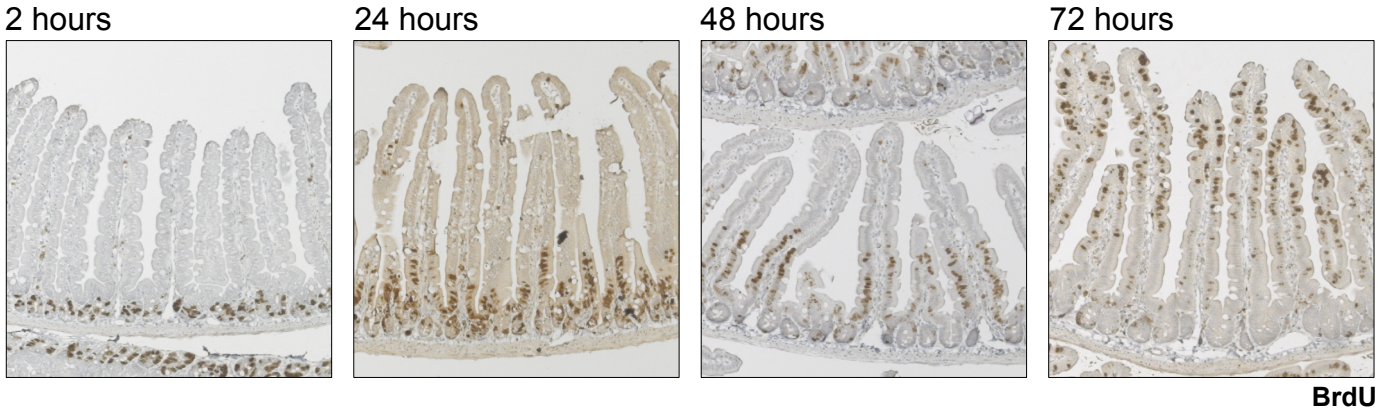


Figure S5. Epithelial turnover in control mice. BrdU immunohistochemistry showing small intestinal epithelial cell turnover 2, 24, 48, and 72 hours after BrdU injection.

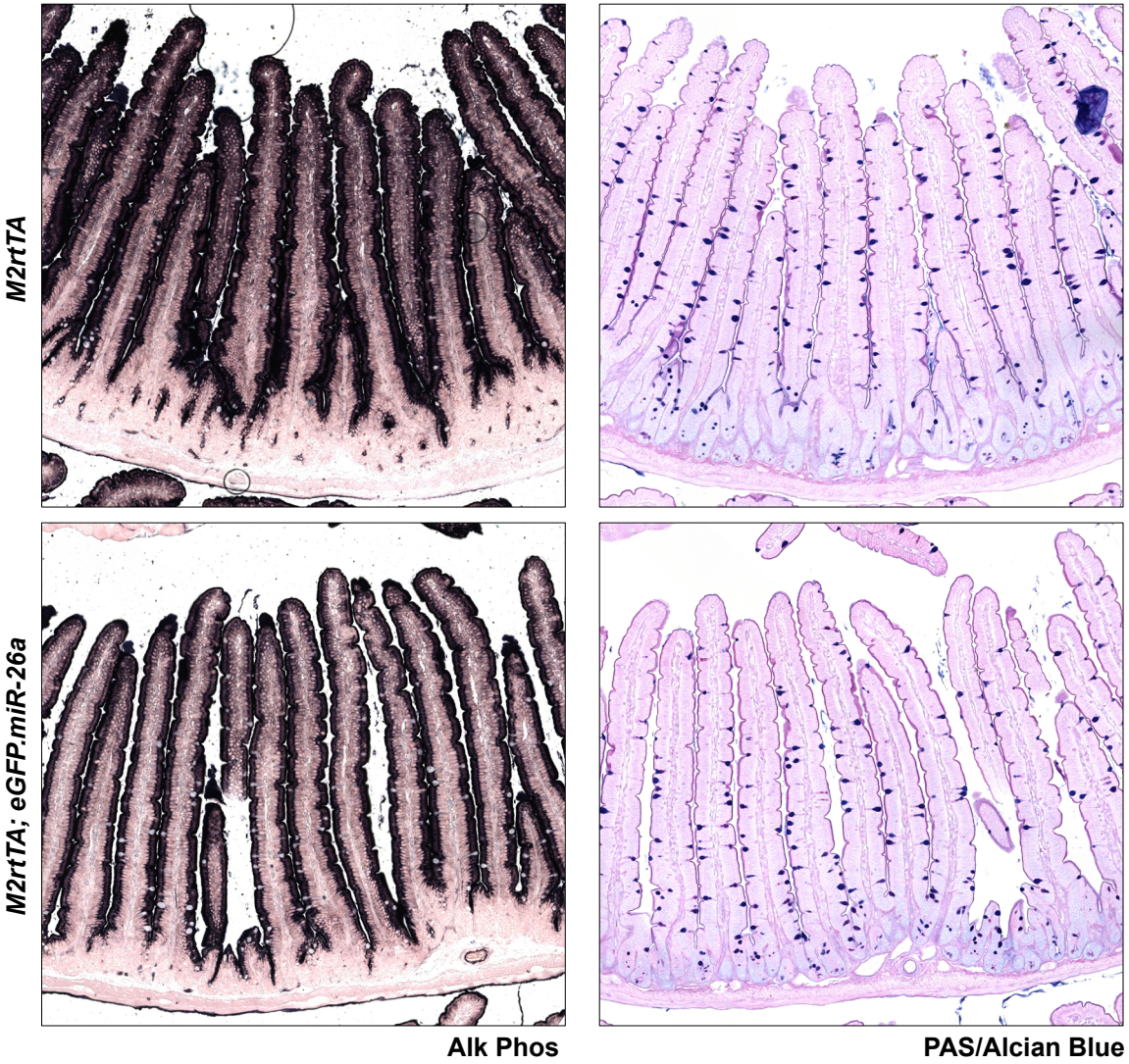


Figure S6. Grossly normal differentiation of intestinal epithelium. Alkaline phosphatase and PAS/Alcian Blue staining of small intestine from dox-treated mice of the indicated genotypes showing overtly normal intestinal epithelial development.

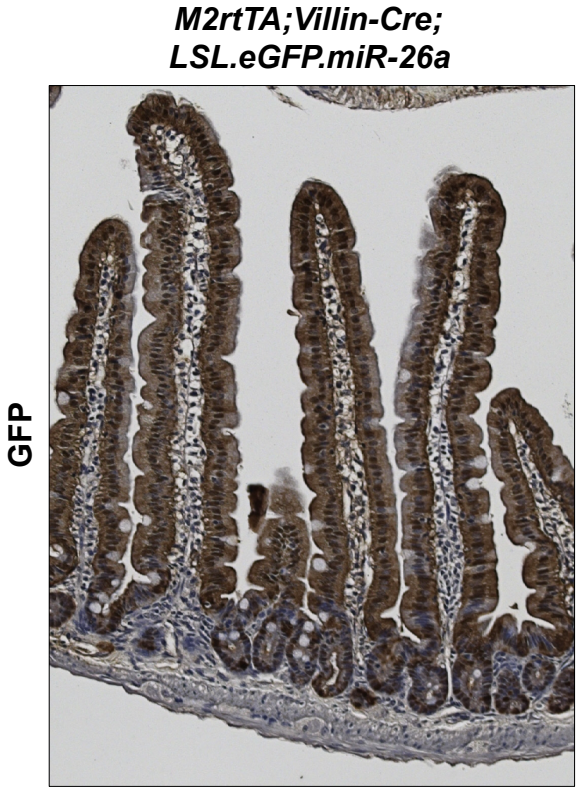


Figure S7. Intestinal epithelium-specific miR-26 transgene expression. GFP immunohistochemistry in small intestine of a *M2rtTA; Villin-Cre; LSL.eGFP.miR-26a* transgenic mouse demonstrating epithelial-restricted GFP expression.

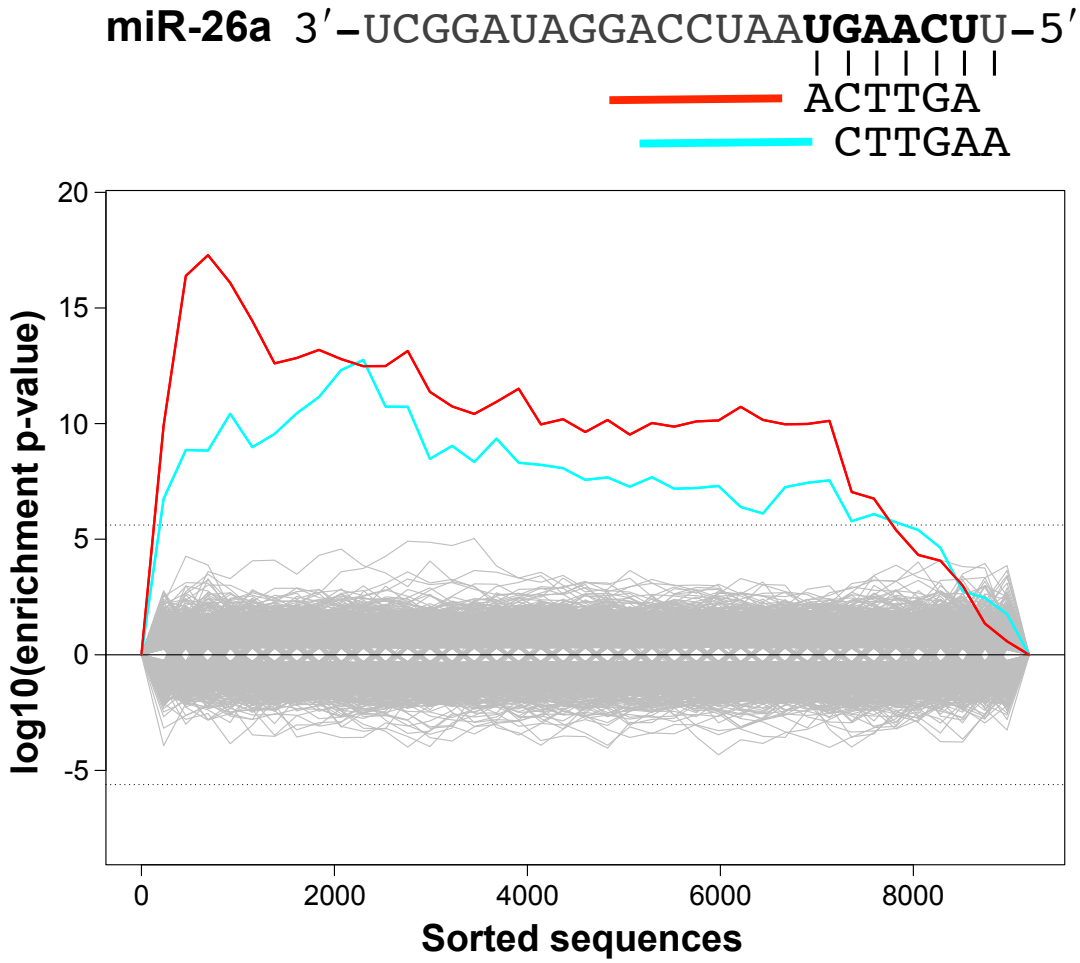
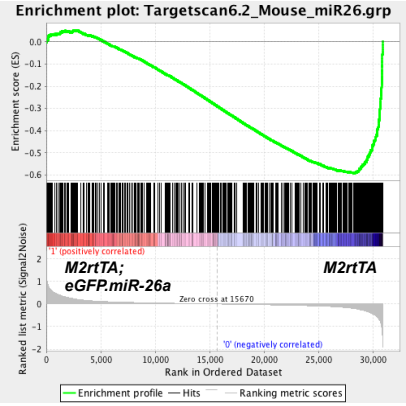


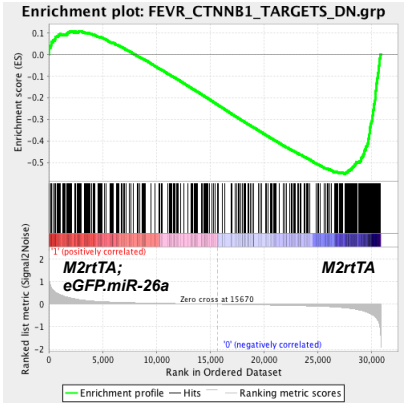
Figure S8. Sylamer analysis of enriched hexamers in transcripts that are repressed in miR-26a transgenic intestinal epithelium. Both significantly enriched motifs correspond to binding sites for the miR-26 seed sequence (alignment shown above Sylamer plot).

A



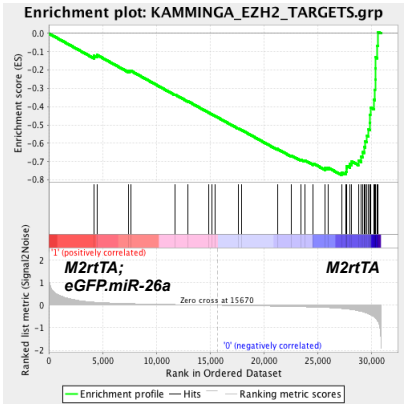
NES = -2.39
p < 0.001
FDR q-value = 0.0
1000 gene set permutations

B

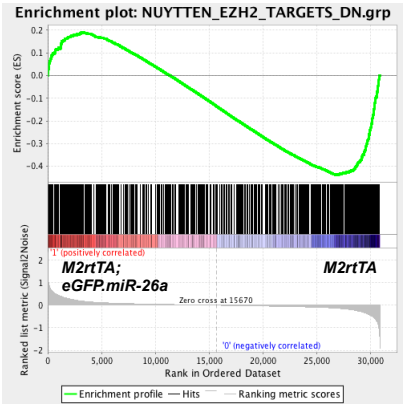


NES = -2.18
p < 0.001
FDR q-value = 0.0
1000 gene set permutations

C



NES = -2.19
p < 0.001
FDR q-value = 0.0
1000 gene set permutations



NES = -1.80
p < 0.001
FDR q-value = 0.0
1000 gene set permutations

Figure S9. Gene Set Enrichment Analysis (GSEA) on microarray data from *M2rtTA* and *M2rtTA; eGFP.miR-26a* intestinal epithelium.

A. GSEA using a custom gene set consisting of all Targetscan 6.2 mouse miR-26a targets.

B. GSEA using an intestinal crypt β-catenin target gene set (FEVR_CTNNB1_TARGETS_DN) from the Molecular Signatures Database (http://www.broadinstitute.org/gsea/msigdb/index.jsp).

C. GSEA using two annotated EZH2 target gene sets from the Molecular Signatures Database (KAMMINGA_EZH2_TARGETS and NUYTTEN_EZH2_TARGETS_DN).

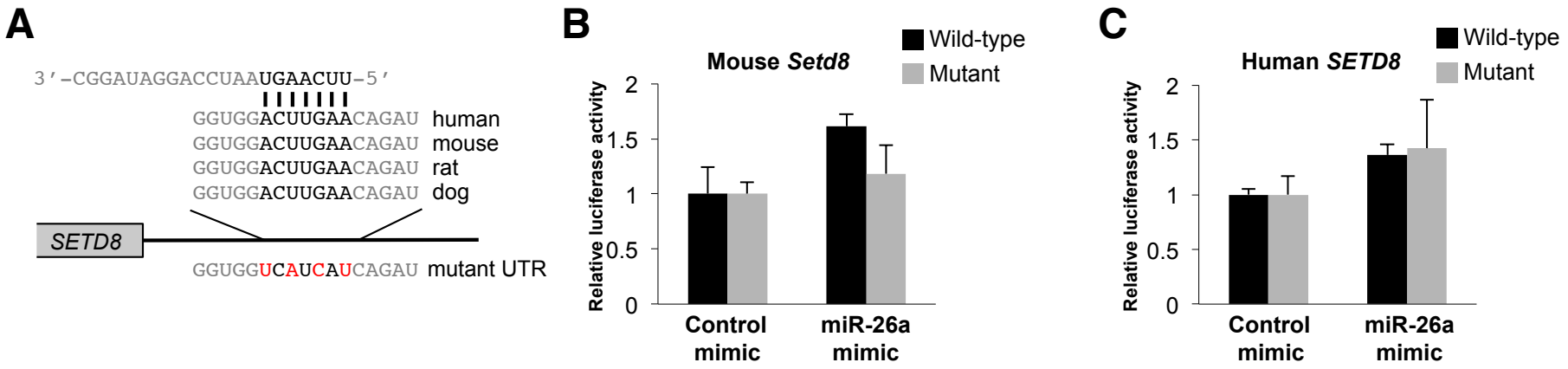


Figure S10. A second predicted miR-26 binding site in the 3′ UTRs of the human and mouse *SETD8* does not mediate repression in luciferase assays.

A. Nucleotide sequence and conservation of the predicted site. Mutations introduced into reporter constructs are shown below the alignment and highlighted in red.

B,C. Relative firefly luciferase activity of wild-type or mutant reporter constructs following transfection into HCT116 cells with control or miR-26a mimic. n=3 replicates per condition.

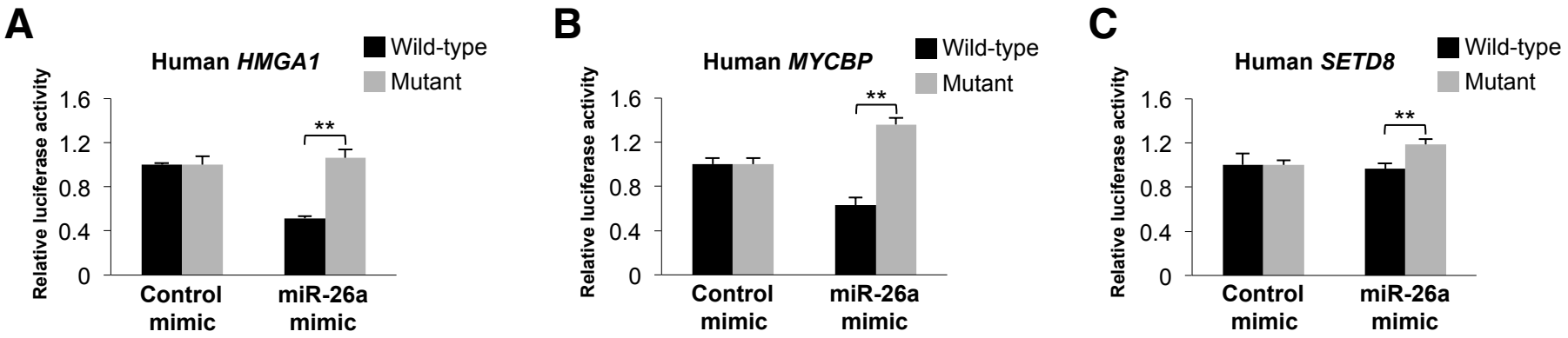


Figure S11. Validation of miR-26 binding sites in the 3′ UTRs of the indicated human transcripts. Nucleotide sequence of each site is shown in Figure 5C,E,G. Graphs show relative firefly luciferase activity of wild-type or mutant reporter constructs following transfection into HCT116 cells with control or miR-26a mimic. n=3 replicates per condition. **, $p<0.01$ (2-tailed t-test).